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ATTRACTANT PHEROMONE FOR MALE RICE BUG, *Leptocorisa chinensis*: SEMIOCHEMICALS PRODUCED BY BOTH MALE AND FEMALE

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Abstract—GC profiles of the airborne volatiles produced by adult males and females of the rice bug, *L. chinensis* showed no qualitative chemical dimorphism. However, GC-EAD experiments showed that eight of the compounds elicited strong responses in male and female antennae. Ruling out alarm pheromone and compounds that were not found in the whole-body extracts of the bugs, four compounds remained to be tested as possible attractants. In field experiments, the whole mixture or tertiary blends were not attractive; however, males were strongly attracted to a 5:1 mixture of 2-(*E*)-octenyl acetate and octanol. The attractancy of the binary mixture was decreased by the addition of 3-(*Z*)-octenyl actetate. Although the binary lure specifically attracted males, there was no evidence that it triggered any response of sexual behavior in males.

Key Words—Heteroptera, Alydidae, GC-EAD, 2-(*E*)-octenyl acetate, octanol, 2-(*E*)-octenal, 2-(*E*)-octenol, octyl acetate, 3-(*Z*)-octenyl acetate, rice bug, *Leptocorisa chinensis*

INTRODUCTION

There are some 35,000 species of Heteroptera worldwide, yet sexual pheromones are fully known only for a few species. The diverse nature of the group

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itself has hindered progress, and the copious defensive secretions from metathoracic glands found in most true bugs can contaminate sex pheromone samples (Aldrich, 1995). In order to alleviate the problem derived from their overwhelming chemical fortification, we have used gas chromatography coupled with an electroantennographic detector (GC-EAD) to study chemical communication in the bean bug, *Riptortus clavatus*. This technique allowed us not only to separate defensive substances from semiochemicals but, more importantly, to isolate an alarm pheromone (Leal and Kadosawa, 1992) from other attractants. Further studies led to the identification of male-specific multifunctional semiochemicals that play the double role of attractant pheromone for adults as well as aggregation pheromone that enables second instars to find the host food plant (Leal et al., 1995). This prompted us to investigate chemical communication of other species of the same family (Alydidae). In that connection, the rice bug, *Leptocorisa chinensis* (Dallas), *kumoherikamemushi* in Japanese, is an interesting case study because it is an economically important pest in Japan, China, and other South East Asian countries for which alternative methods of control are badly neeeded. Surprisingly, we found that males and females of the rice bug produce various semiochemicals that are sensed by both sexes, two of them being attractive only to males.

METHODS AND MATERIALS

Analytical Procedures. Gas chromatography (GC) was carried out with a Hewlett-Packard 5890 II Plus instrument equipped with a split/splitless injector, electronic pressure control, a flame ionization detector, and an HP 3365 Series II Chemstation. High-resolution GC analyses were performed with polar and nonpolar capillary columns: HP-Innowax and HP-5MS (30 m $\times$ 0.25 mm $\times$ 0.25 μm; Hewlett-Packard), respectively. These columns were operated at 70°C for 1 min, increased to 230°C at a rate of 10°C/min, and held at this temperature for 10 min. Low-resolution mass spectrometry (MS) was carried out with an HP 5890 II Plus gas chromatograph linked to a mass selective detector MSD 5972 (Hewlett-Packard). Chromatographic resolution was achieved on a HP-Innowax column operated under the same conditions as described above. MS were also obtained on an Hewlett-Packard gas chromatograph electron ionization detector GCD Series, equipped with an HP-5MS capillary column that was operated at 50°C for 1 min, increased to 150°C at a rate of 5°C/min, held at this temperature for 1 min, increased to 230°C at a rate of 10°C/min, and finally held at this temperature for 10 min.

Gas Chromatography with Electroantennographic Detector (GC-EAD). The responses of *L. chinensis* antennae were recorded with a GC-EAD system (Struble and Arn, 1984). An HP 5890 Series II Plus Gas chromatograph was modified to have the effluent from the capillary column split between EAD and FID (3 : 1 ratio). Bug antennae were placed in a previously described acrylic stage (Leal et al., 1992) which was set inside the glass transfer line (2 cm away from the GC outlet) and connected with gold wires to an amplifier (gain 5). The signal was filtered through a passive filter (cutoff frequency 0.12 Hz) and fed into an A/D 35900E interface (Hewlett-Packard). Simultaneous acquisitions of FID and EAD signals were obtained with the above chemstation.

Extracts. For the collection of airborne volatiles, field-collected adult bugs, separated into male and female groups, were anesthetized with CO_2 to minimize the release of defensive secretion and transferred to an all-glass aeration apparatus. The aeration apparatus contained a piece of wet cotton and an ear of foxtailgrass, *Setaria viridis* (Gramineae). The chamber was placed inside an incubator maintained at 25°C and a 16L : 8D photoperiod. Volatile compounds were trapped on a Super Q (Alltech, Deerfield, Illinois) column. The trapped compounds were eluted by washing the column with hexane and the extract was concentrated to 0.1 insect equivalent/μl. Whole-body extracts were obtained by immersing at least three individuals in hexane for 3 min.

Chemicals. 2-(*E*)-Octenyl acetate and 3-(*Z*)-octenyl acetate were prepared by the reaction of the respective alcohols with acetyl chloride in dichloromethane. Octyl acetate, 2-(*E*)-octenol, and octanol were purchased from Tokyo Chemical Industry Co. Ltd.

Traps and Field Tests. Newly developed sticky traps (20 × 20 cm) were made of waterproof paper coated on both sides with adhesive polybutadiene. Field experiments were conducted in a rice field inside Ibaraki Research Center in the summer of 1995. Chemicals (1 mg) were incorporated in plastic pellets (4–5 mm in diameter) made of polyethylene–vinyl acetate. Ten pellets were loaded on the sticky plate traps, and tests were run with the traps distributed around a rice field with three replicates. Traps were placed at 25 cm above the ground with an intertrap distance of 10 m. Capture data were collected daily for at least three consecutive days; these data were transformed to log $(x+1)$ before difference among means were tested for significance by ANOVA with the JMP Software, Version 2 (Anonymous, 1989). Treatments followed by the same letters are not significantly different at the 5% level in the Tukey-Kramer honestly significant difference test.

RESULTS AND DISCUSSION

The GC-EAD profile of the airborne volatiles collected from *L. chinensis* females showed the occurrence of at least eight peaks that were EAD-active (Figure 1). The same EAD responses were obtained with male or female antennae as the sensing element.

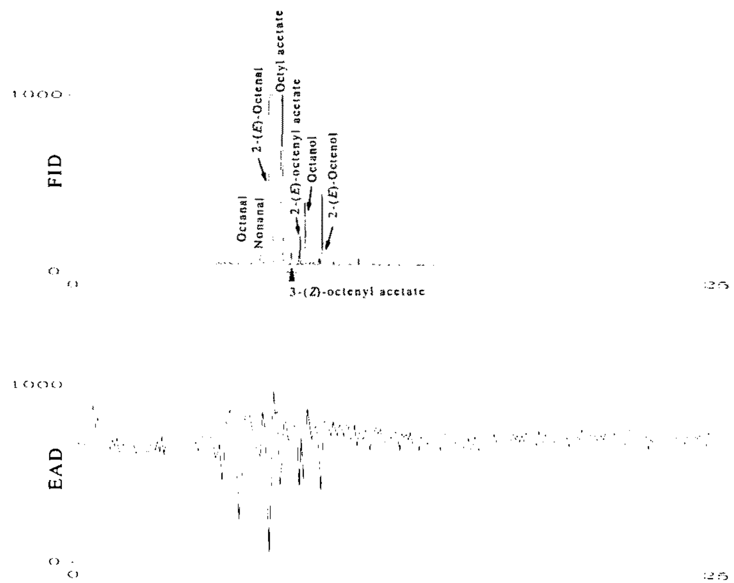

FIG. 1. Parallel FID and EAD chromatograms obtained from airborne volatile of *L. chinensis* females during the scotophase (0.1 female equivalent) and separated on an HP-Innowax column. The EAD profile using a female antenna as the sensing element (in the figure) was identical to the profile obtained with male antenna (not shown).

Interestingly, these EAD-active peaks were detected in the extracts from males as well as from females not only in the scotophase (Figure 2), but also in the photophase. The FID profiles showed only quantitative differences. There was no qualitative difference in the whole-body extracts of males and females.

The EAD-active peaks were identified by their mass spectra, followed by comparison with authentic compounds that gave the same retention times as the natural products on both polar and nonpolar columns. Because the bugs released abundant amounts of 2-(*E*)-octenal when disturbed, this compound was considered to be part of the alarm pheromone system. In fact, 2-(*E*)-octenal has been previously identified as an alarm pheromone of other hemipterans (Blum, 1985). Toxicity and repellency tests also showed that 2-(*E*)-octenal is part of the defensive secretion of the rice bug, *L. oratorius* Fabricius (Gunawardena and Bandumathie, 1993). Octanal and nonanal were ruled out as part of an attractant pheromone since these compounds were not found in the whole-body extracts.

Four compounds remained to be tested as possible attractants for the adult bugs: octyl acetate (OAc), 2-(*E*)-octenyl acetate (2EOAc), octanol (OL), and

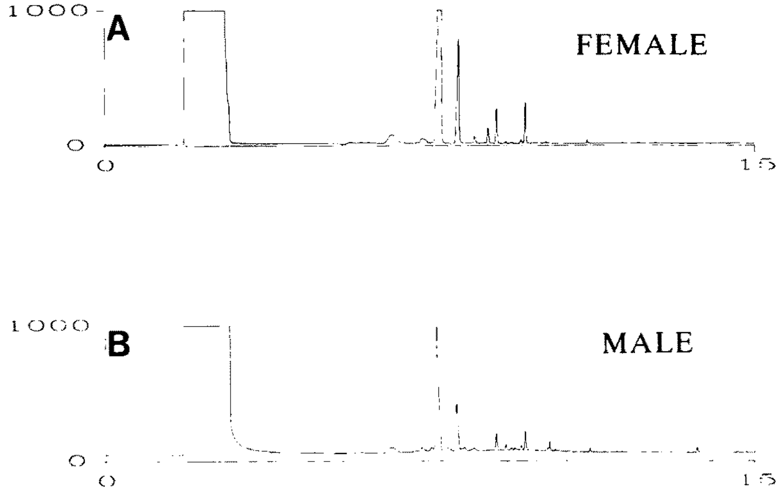

FIG. 2. GC profiles of the airborne volatiles of (A) female and (B) male *L. chinensis* collected during 6 hr of the scotophase.

2-(E)-octenol (2EOL). The activity of these compounds was tested in the field by utilizing the ratio typically found in the airborne extracts; the ratio of 2EOAc, OAc, 2EOL, and OL was 10:5:2:2. This is not the ratio of the sample shown in Figure 1. Preliminary experiments showed that captures in traps baited either with a full mixture or with tertiary mixtures were not significantly different from catches in control traps.

Finally, the attractants were identified by testing binary combinations of the four compounds. These experiments revealed that a mixture of 2EOAc and OL was very attractive to males (Figure 3).

Catches in traps baited with 2EOAc and 2EOL, albeit small, were also significantly higher than captures in control traps. Further experiments involving only two treatments confirmed that males were attracted significantly more to the 2EOAc + OL lure than to the 2EOAc + 2EOL; the latter also captured significantly more bugs than control traps (Figure 4). The results were clear-cut because there was no catches at all in control traps throughout these experiments. In the preliminary tests, however, bugs were captured in control traps, probably due to the effect of weeds in the vicinity of the traps. This interference was completely eliminated by cleaning the rice field before the next series of experiments.

It was found that both male and female *L. chinensis* also produce 3-(Z)-octenyl acetate (3ZOAc), although EAD response to this compound was very

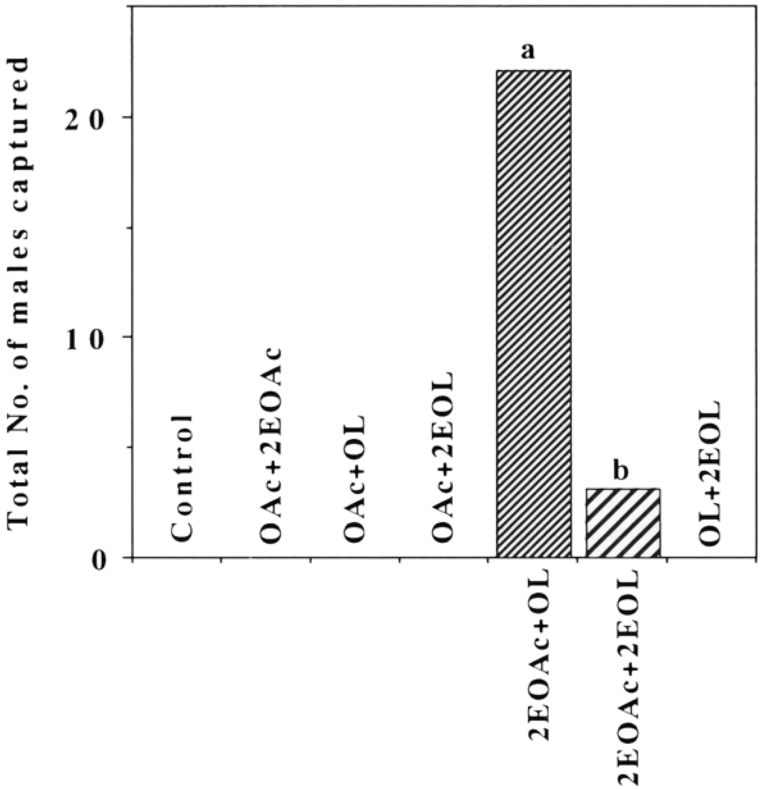

FIG. 3. Catches of *L. chinensis* males in sticky traps baited with binary combinations of the four possible attractants.

weak. Field tests showed that this compound decreased the captures of male rice bug in traps baited with the attractant mixture, 2EOAc + OL (Figure 5). Whether 3-(*Z*)-octenyl acetate is a part of the alarm pheromone system of the rice bug or a behavioral antagonist is yet to be clarified. Furthermore, 2EOL seems to have negative effects on the catches, but further experiments must be carried out to determine the role of this semiochemical in the chemical communication of the rice bug.

Sex pheromones have traditionally been considered by researchers to be those compounds that are emitted by individuals of one sex and that cause attraction of members of the opposite sex, resulting in the location of the emitter, and subsequently, mating (Baker, 1989). Contrary to many other families, sex pheromones of hemipterans are largely produced by males. It is worth noting that in alydids, the metathoracic scent glands are sometimes sexually dimorphic,

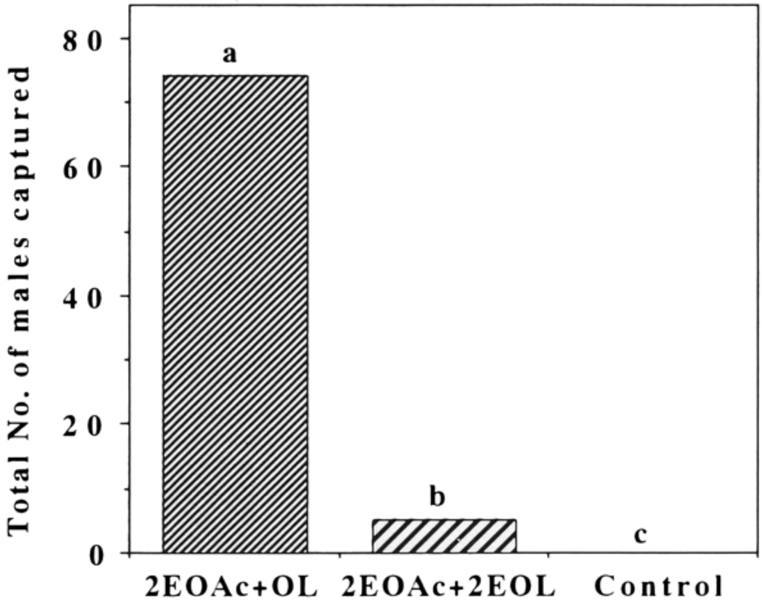

FIG. 4. Captures of males of the rice bug in sticky traps baited with 2-(*E*)-octenyl acetate plus octanol and 2-(*E*)-octenyl acetate plus 2-(*E*)-octenol.

and the dimorphisms are expressed chemically (Aldrich et al., 1993). Recently, we found that males of the bean bug *Riptortus clavatus* release semiochemicals that attract both males and females (Leal et al., 1995). Interestingly, the case of the rice bug is just the opposite, i.e., only males are attracted to semiochemicals that are produced and released by both males and females. Although female antennae of *L. chinensis* can "smell" the male attractants (and the other semiochemicals tested), they do not elicit any attraction for females. Throughout the whole series of experiments, only four females were captured, although insect samples collected in the same field had a sex ratio nearly 1 : 1.

In conclusion, 2EOAc and OL are produced by both male and female of *L. chinensis* and attract only males of the same species. Field experiments showed that a 5 : 1 mixture is very attractive, but the optimum ratio of the attractants is yet to be determined.

For this attractant pheromone we avoided using the term sex pheromone (Tamaki, 1985) because, despite the fact that the chemicals are "secreted by an organism to the outside and cause a specific reaction in a receiving organism of the same species" (Nordlund, 1981), there is no evidence that these semiochemicals trigger any sexual behavior in males other than attraction. Future

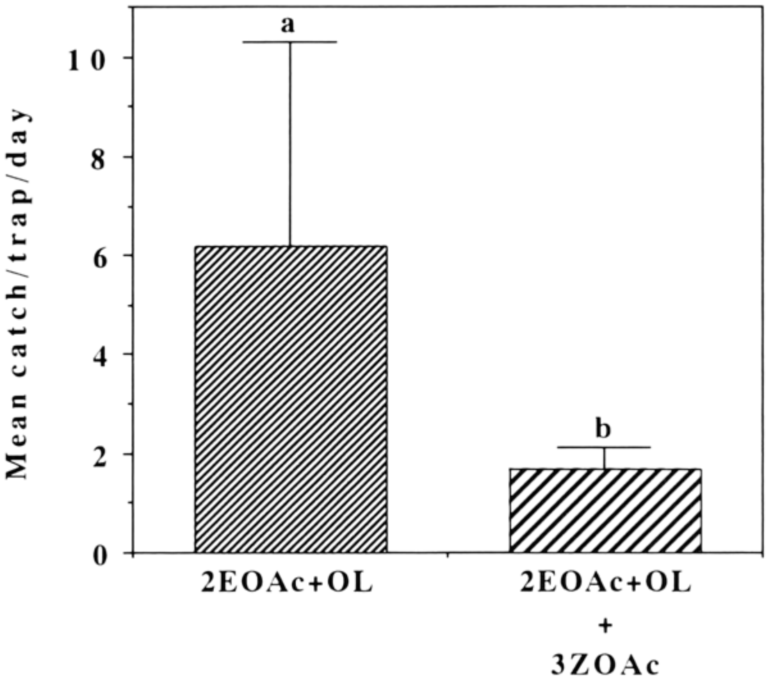

FIG. 5. Captures in traps baited with a 2-(*E*)-octenyl acetate + octanol (5 : 1) and the binary mixture plus 3-(*Z*)-octenyl acetate (5 : 1 : 1).

detailed behavioral studies will be carried out to test the possibility that these semiochemicals are involved in lek formation.

Throughout this paper we used the term semiochemicals (Nordlund and Lewis, 1976) for all EAD-active compounds because the compounds are released and sensed by the same species, and they are likely to be involved in chemical interactions between the rice bugs. Further studies are needed to clarify their specific role in the chemical ecology of this species.

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REFERENCES

ANONYMOUS. 1989. Software for statistical visualization on the Apple Macintosh. JMP introductory guide. SAS Institute, Inc., Cary, North Carolina.

ALDRICH, J. R. 1995. Chemical communication in the true bugs and parasitoid exploitation, pp. 318–363, *in* R. T. Cardé and W. J. Bell (eds.). Chemical Ecology of Insects II. Chapman & Hall, New York.

ALDRICH, J. R., WAITE, G. K. MOORE, C., PAYNE, J. A., LUSBY, W. R. and KOCHANSKY, J. P. (1993). Male-specific volatiles from Neartic and Australasian true bugs (Heteroptera: Coreidae and Alydidae). *J. Chem. Ecol.* 19:2767–2781.

BAKER, T. C. 1989. Sex pheromone communication in the Lepidoptera: New research progress. *Experientia* 45:248–262.

BLUM, M. S. 1985. Alarm pheromones, pp. 193–224, *in* G. A. Kerkut and L. I. Gilbert (eds.). Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol. 9, Pergamon Press, Oxford.

GUNAWARDENA, N. E., and BANDUMATHIE, M. K. 1993. Defensive secretion of the rice bug, *Leptocorisa oratorius* Fabricius, (Hemiptera: Coreidae): A unique chemical combination and its toxic, repellent, and alarm properties. *J. Chem. Ecol.* 19:851–861.

LEAL, W. S., and KADOSAWA, T. 1992. (*E*)-2-Hexenyl hexanoate, the alarm pheromone of the bean bug *Riptortus clavatus* (Heteroptera: Alydidae). *Biosci. Biotech. Biochem.* 56:1004–1005.

LEAL, W. S., MOCHIZUKI, F., WAKAMURA, S., and YASUDA, T. 1992. Electroantennographic detection of *Anomala cuprea* Hope (Coleoptera: Scarabaeidae) sex pheromone. *Appl. Entomol. Zool.* 27:289–291.

LEAL, W. S., HIGUCHI, H., MIZUTANI, N., NAKAMORI, H., KADOSAWA, T., and ONO, M. 1995. Multifunctional communication in *Riptortus clavatus* (Heteroptera: Alydidae): Conspecific nymphs and egg parasitoid *Ooencyrtus nezarae* use the same adult attractant as chemical cue. *J. Chem. Ecol.* 21:973–985.

NORDLUNG, D. A. 1981. Semiochemicals: A review of the terminology, pp. 13–28, *in* D. A. Nordlund, R. L. Jones, and W. J. Lewis (eds.). Semiochemicals: Their Role in Pest Control, Wiley & Sons, New York.

NORDLUND, D. A., and LEWIS, W. J. 1976. Terminology of chemical releasing stimuli in intraspecific and interspecific interactions. *J. Chem. Ecol.* 2:211–220.

STRUBLE, D. L., and ARN, H. 1984. Combined gas chromatography and electroantennographic recording in insect olfactory responses, pp. 161–178, *in* H. E. Hummel and T. A. Miller (eds.). Techniques in Pheromone Research. Springer-Verlag, Tokyo.

TAMAKI, Y. 1985. Sex pheromones, pp. 145–191, *in* G. A. Kerkut and L. I. Gilbert (eds.). Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol. 9, Pergamon Press, Oxford.